STUDIES ON FIBERS IN TISSUE CULTURE III

THE DEVELOPMENT OF RETICULUM INTO COLLAGENOUS FIBERS IN CULTURES OF ADULT RABBIT LYMPH NODES

(WITH FIVE FIGURES AND TWO PLATES)

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

1930

By

ROSCOE LEWIS McKINNEY

THE UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS

Reprinted from
ARCH. FÜR EXPERIMENTELLE ZELLFORSCHUNG
IX: 14–35, 1929

STUDIES ON FIBERS IN TISSUE CULTURE III

THE DEVELOPMENT OF RETICULUM INTO COLLAGENOUS FIBERS IN CULTURES OF ADULT RABBIT LYMPH NODES

(WITH FIVE FIGURES AND TWO PLATES)

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

1930

By

ROSCOE LEWIS McKINNEY

THE UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS

Reprinted from
ARCH. FÜR EXPERIMENTELLE ZELLFORSCHUNG
IX: 14-35, 1929

R. UNIV.
BIBLIOTHEEK
LEIDEN

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41546816_0061

Nachdruck verboten.
Übersetzungsrecht vorbehalten.

(From the Department of Anatomy of the University of Chicago, Chicago, U. S. A.)

Studies on Fibers in Tissue Culture. III.

The Development of Reticulum into Collagenous Fibers in Cultures of Adult Rabbit Lymph Nodes[1]).

By

Roscoe L. Mc Kinney.

With 5 figures and plate 2 and 3.

Introduction.

Although it is generally believed that reticulum fibers can change into collagenous fibers, all of the stages in this process have not been made clear. Some authors claim that collagenous and reticulum fibers are identical and that the apparent differences in their staining reactions are merely due to slight physical differences between them. In the following pages I shall detail some experiments on tissue cultures of rabbit lymph node which throw some light on the relationships between these two types of fibers.

The origin of connective tissue fibers has been extensively studied in embryonic and adult tissues as well as in tissue cultures. There is still a wide divergence in the opinions of various authors as to the mode of origin and nature of the different fibers.

As early as 1876 von Kupffer described certain fibers under the term „Gitterfasern" in the liver. The term "argyrophil fibers" has been applied to them by Schieferdecker (1911) and Schaf-

1) The cost of publishing the plates has been met by the Douglas Smith Foundation for Medical Research of the University of Chicago.

fer (1921), because they are stained black by the Bielschowsky-Maresch silver impregnation method. Mall in 1896 called this same type of fiber "reticulum".

In some of his earlier works Maximow (1902) noted in the cell body of fibroblasts certain long, thin fibrils that stained black with iron hematoxylin; he called them "tonofibrils" as he believed they were similar to the structures described by Heidenhain in other types of cells.

Mallory (1904), using another technique, described the same type of fibrils in fibroblasts; he gave them the name of "fibroglia fibers". It is probable that M. R. Lewis (1917) obtained these tonofibrils in her cultures and not argyrophil fibers. The relationship between fibroglia fibers, mitochondria and argyrophil fibers has not been made clear up to the present time.

Mallory and Parker (1927) believe that reticulum fibers and collagenous fibers are the same, and that the silver impregnation methods are specific for collagenous fibers, but only under certain physical conditions. They are of the opinion that reticulum does not exist as a chemically specific intercellular substance; it is collagen in a separated form and as such can be rendered prominent by the silver impregnation. Reticulum stains the same as collagen with the van Gieson, Verocay, anilin blue and phosphomolybdic hematoxylin methods. This fact Mallory uses to substantiate his claim that reticulum fibers are merely separated collagenous fibers.

Foot (1928) clearly points out, however, that Mallory and Parker's conception of the identity of reticulum and collagenous fibers is untenable for, as he says, "If the substances were chemically identical and their differences in staining merely a matter of physical accessibility to the silver bath, we should expect: that the surface of the section, all of it equally accessible to the fluid, would have a superficial mirror like black coating, which it does not..." Foot recognizes, in the connective tissue frame-work of the spleen, three main groups of fibrous substances, which he can identify by accurate methods of chemical analysis. Such chemical differences, therefore, would allow a selective staining of these various types of connective tissue, if the proper technique is used.

Laguesse (1921) is of the opinion that an amorphous, lamellated, precollagenous, ground substance appears in young mammalian embryos and that it is formed at the expense of interlacing processes of mesenchyme cells, that is, from an exoplasm.

The connective tissue fibrils appear early in this pre-collagenous ground substance as a poorly defined, irregular network. As they grow they gradually lose their free anastomoses; each fibril grows, undergoes secondary cleavages and thus becomes longitudinally striated. New bundles of these fibers are formed and in course of time they acquire the characteristics of collagenous fibers. But they always remain included in the amorphous, lamellated, pre-collagenous, ground substance which serves as a sheath to the bundles and as a cement uniting the individual fibrils.

Baitsell (1925) states, "That in chick and amphibian embryos, the fore-runner of connective tissue is a transparent, gelatinous, cell-free, ground substance, which appears prior to the advent of the mesenchyme cells. This ground substance is a secretion of the cells of the various germ layers and not as Szily (1907) says, 'a syncitium of the processes of the cells of the germ layers, a sort of exoplasm'." Baitsell believes the fibers develop in the ground substance by changes in the ground substance itself. Harrison (1925) has the same point of view as Baitsell on the origin and development of connective tissue fibers.

Nageotte (1922) in an extensive series of experiments has brought much evidence in favor of the view that the fibers arise between the cells and that at no time are they connected with the cells. He denies the existence of a living ground substance and believes that the fibers develop at the expense of fibrin or fibrin-like structures.

Baitsell (1915) found that in two and three day cultures of adult frog connective tissue, small and indistinct, fiber-life structures appear in the plasma clot surrounding the explanted tissue. These fibers have an index of refraction different from that of fibrin. They increase in size and ramify in all directions through the clot. He believes that the fibers are not direct outgrowths of the explanted tissue but are formed from the fibrin in the presence of living cells. Three types of cultures were made to demonstrate this: serum plus explant, plasma clots without explant, and plasma clots plus starch granules and dead corpuscles; in each case no fiber formation took place. In cultures ranging from 5 to 14 days of incubation he found that the plasma clot and the new fibers were digested by pancreatin. This, he believed, indicated that the fibers were fibrin-like in character.

M. R. Lewis (1917) washed bits of subcutaneous connective tissue, deep fascia and arachnoid membrane of chick embryos in

several changes of Locke's solution and then explanted them in a medium free from fibrin. After 24 hours' growth she found that fibrils appear as slightly refractive lives within the cytoplasm of individual cells. Mitochondria were often stretched along these lines but they did not take part in the formation of the fibrils. In 48 and 72 hour cultures the refractive lines of more primitive fibrils appear and become partly gathered in bundles. The cytoplasm of the cells at this time becomes separated into exoplasm and endoplasm. The endoplasm is granular and contains mitochondria, fat and granules that stain with neutral red immediately around the nucleus; the exoplasm on the other hand is the clear, non-granular surface of the cell. Fibrils gradually appear in the exoplasm; in the course of development they become more and more definite and independent of the cytoplasm, although they can always be traced into a cell.

Maximow (1928, 1929) found a fibrous intercellular substance in cultures of adult thymus and blood leucocytes explanted in the usual plasma and embryonic extract medium. He saw isolated rod and spirochete-shaped structures which stained black with silver and fused to form the first fibers. These formed a delicate, loose network of branching and anastomosing fibrils. In many places they followed the outline of the fibroblasts and their processes and were closely adherent to their protoplasm. The fibrils were seen from the beginning to extend far into the surrounding medium. Maximow noted further that the outline of the fibroblasts was always distinct, and that the existence of an exoplasm could be excluded with certainty. The fibrils sometimes seemed to follow the course of the threads of the fibrin net-work. He could not prove the transformation of the latter into argyrophil fibers. He observed, in the older cultures, that the argyrophil fibers changed into collagenous fibers.

In 12 day cultures of thymic tissue, Maximow found reticulum fibers; in 25 day cultures he noted that typical collagenous fibers began to appear. In blood leucocyte cultures he found that collagenous fibers appear after from 40 to 50 days.

In view of the striking results obtained by Maximow, a systematic study of the development of fibers in tissue culture has been undertaken in this laboratory. In this paper will be presented the results of a study of cultures of mesenteric lymph nodes of adult rabbits for the very early stages of the transformation of argyrophil fibers into collagenous fibers.

Materials and Methods.

The mesenteric lymph node was aseptically excised from the body of a recently killed adult rabbit. The node was divided into two portions; one was immediately fixed in Zenken-formol to be used as a control, the other was minced into pieces, about one-half of a milimeter in diameter, to be used as explants in the cultures. The cultures were made on small round cover-slips according to Maximow's method. For the culture medium, a mixture of equal parts plasma taken from an adult, non-pregnant rabbit and rabbit embryonic extract obtained from 4 cm. embryos was used.

Some control cultures were made of rabbit plasma and embryonic extract without any tissue; these were incubated for 14 days. Portions of rabbit embryonic extract, adult rabbit plasma and a mixture of the two were fixed separately with Zenker-formol to be used as additional controls.

The cultures were examined daily in vivo. Some were fixed 5 hours after explantation; others were fixed each day. The oldest cultures were those allowed to grow for 11 days. On the sixth day all cultures were transplanted. The method of transplantation was to immerse the individual cultures, with the round cover slips to which they adhered, in a bath of Tyrode's fluid for twenty minutes; fresh diluted embryonic extract was then added to the washed cultures.

The method of embedding and sectioning employed was that of Rubaschkin-Maximow. Some of the preparations were fixed, stained and mounted in toto in damar.

Staining of in toto Preparation.

The method used here is the rapid method of Foot and Mènard (1927) for the silver impregnation of reticulum. The time, as given by them, has been modified to that which I found necessary for successful impregnation. This technique of silver impregnation is specific for reticulum fibers and is a modification of Bielschowsky's original method for the demonstration of the axis-cylinders of nerves. I am giving here in detail the method of Foot and Mènard as I employed it.

A fresh solution of silver-ammonium carbonate was prepared for each batch of cover slip cultures. "To 10 cc. of a 10% aqueous solution of silver nitrate, is added 10 cc. of a saturated solution

of lithium carbonate, distilled water being used for both. The resulting heavy white precipitate of silver carbonate is allowed to settle and the supernatant fluid poured off. The precipitate is then washed several times in from 25 to 50 cc. of distilled water, allowed to settle each time and the supernatant fluid decanted. After 25 cc. of fresh distilled water is added, this washed precipitate is almost dissolved in strong ammonia water, which is added drop by drop while the container is shaken continuously. The precipitate turns grayish brown as the ammonia is added. About 8 to 15 drops of ammonia will be needed, depending on its concentration. Extreme care must be taken not to overstep the end point of solution, it is better to allow a few granules of precipitate to remain undissolved." The entire solution is then made up to 100 cc. with distilled water that has been heated to about 50° C. If the solution appears cloudy it should be filtered before using. To the back of the cover slip cultures a small amount of vaseline was applied and they were floated, culture side down, on the surface of the respective reagents. A tabulation of the procedure follows:

1) Five minutes in 0.25% aqueous potassium permanganate and ten minutes in 5% aqueous oxalic acid, with a wash of distilled water in between.

2) Twenty minutes in silver-ammonium carbonate at about 50° C.

3) Wash in distilled water and reduce in 20% neutral formalin for two minutes. Rinse well at tap.

4) Tone in 1:500 gold chloride solution for two minutes.

5) Wash and fix for two minutes in 5% sodium hyposulphite.

6) Wash well with distilled water, remove the vaseline from the back of the cover slip, dehydrate by passing through graded alcohols (culture side up), clear in xylol and mount in damar.

The in toto preparation were not counter stained.

Staining of Serial Sections.

All the cultures that were serially sectioned were stained, as indicated above, with Foot's silver impregnation method and counter stained by Heidenhain's modification of Mallory's connective tissue stain (Azan).

The azo-carmin was made up in a 0.25% aqueous solution which was acidified by adding 1 cc. of glacial acetic acid to

100 cc. of the solution. This mixture was heated up to 56° C. before using. A tabulation of the procedure follows:

1) In anilin alcohol (1:1000 using 95% alcohol) for 15 minutes.

2) In 0.25% aqueous solution of azo-carmin acidified with acetic acid for 15 minutes at 56° C.

3) Wash in distilled water.

4) Differentiate in anilin alcohol (as in 1) until the nuclei are reddish yellow.

5) In acid alcohol (100 cc. of 95% alcohol + 1 cc. of glacial acetic acid) for 1 minute.

6) In 5% phosphotungstic acid for 15 minutes.

7) In anilin blue — orange G — acetic acid for 15 minutes.

8) Wash rapidly in distilled water.

9) Differentiate in 95% alcohol.

10) Dehydrate rapidly in absolute alcohol, clear in xylol and mount in damar.

Observations of Living Cultures.

Nothing was seen of the fibers which the special histological methods later showed were present in the living cultures. One of the most striking features of the cultures is the fact that such great numbers of fibers could be present without being seen in the living cultures. The material was not submitted to any intra vitam staining methods as previous studies had not demonstrated fibers by this method.

In the first 24 hours a number of cells migrate from the explant. They are small round cells with very fine processes (lymphocytes). After 48 hours the migrating cells include a number of large, irregulary shaped cells with much protoplasm (macrophages). In some of the culture a very fine, delicate net-work of fibrin can be seen. In 72 hour cultures some of the cells have become elongated and have long outstretched processes. On the fourth day the cultures exhibit many large elongated cells, some with extensive processes. These cells form a compact zone of new growth around the explanted tissue. Many cells in this zone have retained the appearance of lymphocytes and macrophages. I am unable to distinguish any structures in the plasma clot.

On the fifth day the cultures show large areas of liquefaction about the explant and fibroblasts have grown around these areas.

Cultures 6 days old show a zone of outgrowth consisting largely of fibroblasts; these form a dense mass radiating from the explanted tissue.

In cultures ranging from 6 to 11 days, and having had one transplantation on the sixth day, not much detail can be made out in the explant. There is an extensive zone of growth consisting predominantly of fibroblasts with long processes. Many of the cells in this area are large and irregular in shape and contain lipoid droplets.

I cannot recognize any changes in the fibrin of any of the living cultures.

Description of Fixed Material.

Controls.

1) Adult rabbit mesenteric lymph node.

Control sections of the adult rabbit mesenteric lymph node, from which the cultures were made, were stained by Foot's modification of Bielschowsky's method. These exhibit only a network of reticulum fibers and some bundles of collagenous fibers; the former staining black and the latter brownish. (Fig. 1.)

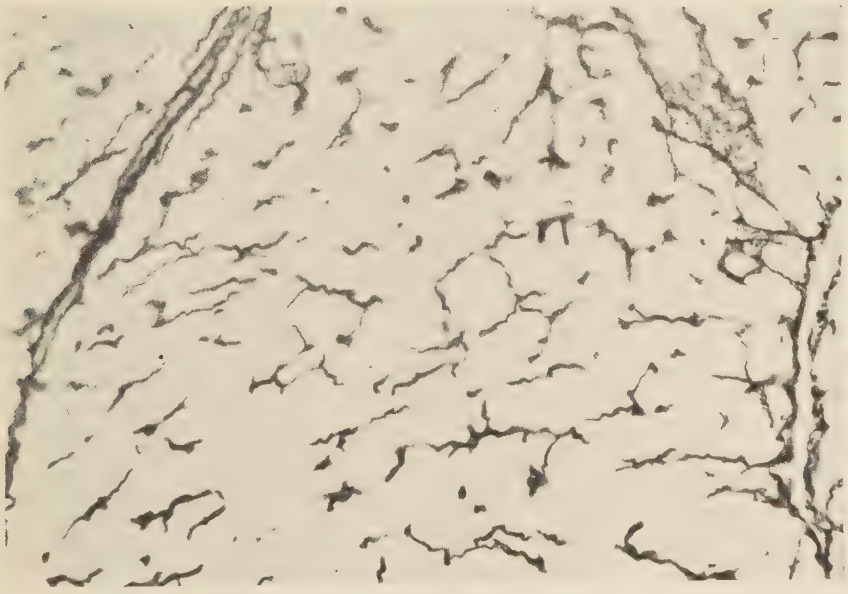


Fig. 1. Control section of adult rabbit lymph node reticulum fibers prior to explantation. Foot's silver impregnation method. 640×.

The collagenous fibers form a capsule and trabeculae extending into the gland. The black reticulum fibers are arranged in a lace-like network between the few, scattered trabeculae. In sections counterstained with hematoxylin-eosin-azure II or "Azan", many smaller lymphocytes, some lymphoblasts (hemocytoblasts) and reticulum cells can be seen in the meshes of the network of reticulum fibers.

With the "Azan" stain the thick collagenous bundles stain a deep blue. The reticulum fibers are in the form of connected, discrete strands forming a meshwork (fig. 1, plate 2). The collagenous fibers are confined to the capsule and trabeculae of the node and constitute wavy bundles.

2) Adult rabbit plasma, rabbit embryonic extract, and a mixture of adult rabbit plasma and rabbit embryonic extract were fixed separately, embedded in celloidin, sectioned and stained by Foot's method. These liquids were so treated to determine whether any argyrophil material was explanted in the culture medium. I find no argyrophil fibrils in the sections.

3) Plasma plus extract cultures explanted without tissue.

A control series of cultures of the rabbit plasma and rabbit embryonic extract that were used in making the lymph node cultures was explanted without any tissue. These were incubated for 14 days with one transplantation on the sixth day. In all respects these were treated as the other cultures, except that they were allowed to incubate longer. Upon microscopic examination the fixed and stained serial sections show no cells. They present a more or less homogenous, grayish blue, finely granular appearance. In some places a fine network of fibrin can be seen. In some of the sections of these control cultures there are a number of short rod shaped and spirochete-like, black, silver stained structures. The spirals, when present, are closely set and irregular. In some cases the structures are coiled. Often they are arranged in an asteroid fashion about some particle of lint. Frequently, however, they appear isolated in regions of the fibrin noticeably free of particles, such as lint, that may have entered the cultures during their preparation. The fibrin about these structures does not appear to be modified other than staining a somewhat deeper blue than elsewhere. These peculiar, fibrillar structures are isolated and neither branch nor anastomose. Their nature is unknown.

Maximow described structures that appear to be similar to these. He found them in very great numbers in his thymus cul-

tures where they extended throughout the fibrin. He described their fusion and believed them to be the first stages in the extracellular formation of the argyrophil fibers. These structures in my cultures are very few in number and never attain the size, or form the intricate network as did the fibrils pictured by Maximow.

In toto Preparations.

All of these preparations were stained by Foot's modification of Bielschowsky's silver impregnation method.

1 and 2 Day Cultures.

The bit of explanted tissue is stained black by the silver. The thickness of the tissue obscures all details of its structure. The fibrin immediately about the explant is clear and into it have wandered a number of cells. With the silver stain alone no cytological details can be made out in them. The fibrin has a grayish, granular appearance, and here and there in it are occasional spirochete-like fibrils similar to those described above. The border of the explanted tissue is sharp.

3 Day Cultures.

Within the explanted tissue the reticulum network is clearly visible. It is different from that of the lymph node controls, in that there are smaller fibers in between the meshes of its original network. It seems as if they have separated from the original large fibers and have formed a fine network between them. In the fibrin near the explant are some highly refractile lines, some of which are incompetely impregnated and are stippled with fine black dots. These fibrils are most numerous in that part of the culture nearest the cover slip. In some instances the fibrils seem to branch off from the reticulum of the explant and to form a delicate network extending from it. There are many large, irregularly shaped cells with a clear round nucleus and large black granules in their cytoplasm (macrophages) in the zone of new growth; the fibrils are closely associated with them. The fibrils extend outward from the explant in most cases, but a few of them run parallel to its margin.

The development that has taken place between 48 and 72 hours in these cultures is indeed spectacular. In the 48 hour cultures the explant appears as a dense bit of tissue in the fibrin surrounded by migrating cells. In the 72 hours cultures the explanted tissue

is less dense and permits its reticulum fiber network to be clearly seen. Numerous macrophages are in the zone of new growth and unquestionably new, argyrophil fibrils have formed in both the explant and the zone of new growth (Fig. 2).

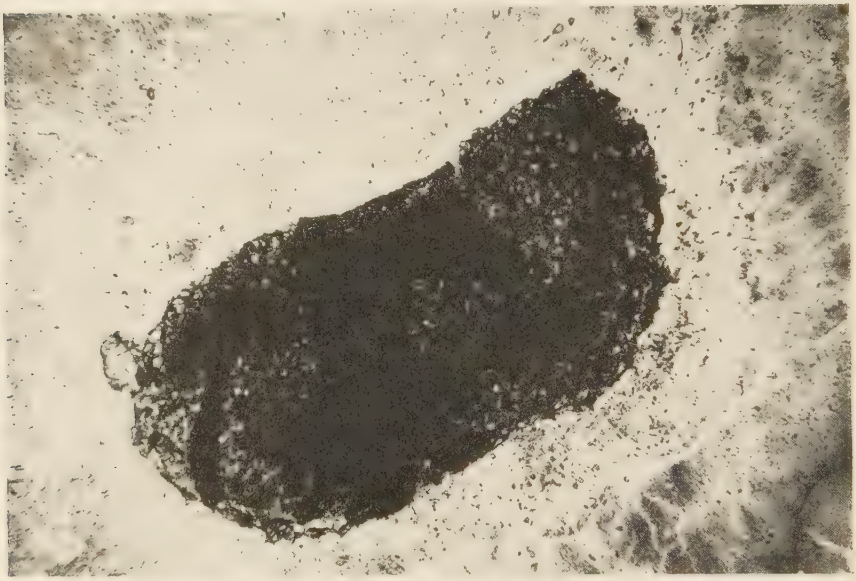


Fig. 2. In toto preparation of a 3 day culture of adult rabbit lymph node. A few thin new argyrophil fibers have appeared in both the explant and new growth, but do not show in the microphotograph. Foot's silver impregnation method. 65×.

4 Day Cultures.

In these cultures there is a great new formation of fibers surrounding the explant. The new fibers radiate from the explant and anastomose frequently. In some cases the fibrils are not uniformly impregnated and appear as broken lines. They are somewhat coarser than the fibrils seen in the 72 hour cultures. There is also a large number of macrophages, some of which divide by mitosis. The new formed fibers seem to be outside of the cell body.

Within the explanted tissue the original, individual, reticulum fibers are wavy and appear to be loose and open. Between the large reticulum fibers is a fine network of fibrils. With a binocular

microscope the new formation of fibers can be seen to have taken place in more than one plane. The newly formed, very fine meshwork in some places is incomplete, so that larger oval spaces devoid of fibers are prominent. I find no fibers at this stage extending beyond the migrating cells (fig. 3).

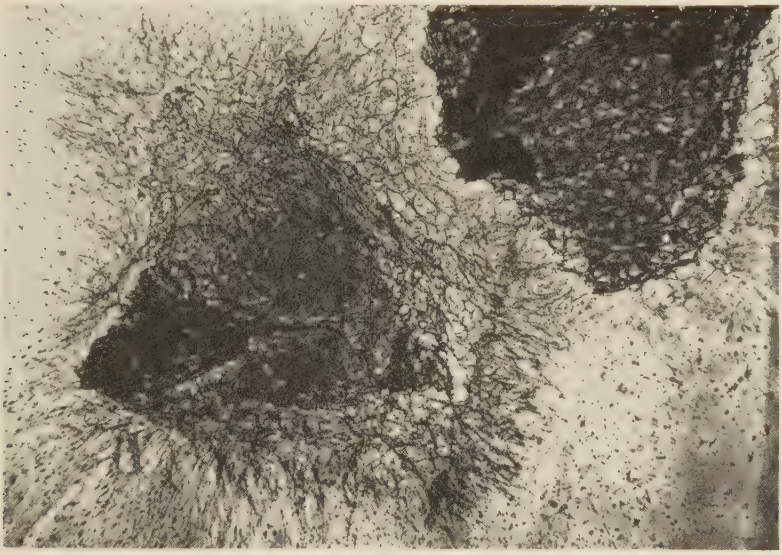


Fig. 3. A 4 day rabbit lymph node culture. In toto preparation. Shows two very dark areas which represent the explanted portions. These are surrounded by the very fine net-work of argyrophil fibers of the new growth. Foot's silver method. 65×.

5 Day Cultures.

In these cultures there is an abundant formation of reticulum fibers within the explant and in the zone of new growth. The new fibers form a dense network about the coarse reticulum network of the explant. Many macrophages and fibroblasts have migrated into the fibrin. In the zone of migrating cells very fine fibrils are found; these anastomose less freely and have a more distinctly radial arrangement than the fibrils in the region immediately around the explant. The fibrils in the zone of migrating cells are very fine and appear as dotted lines. They are near the cover slip. In some cases these fine fibrils extend into the fibrin beyond the migrating cells. In general the fibers pursue a direction radiating from the explanted tissue (fig. 4).

Fig. 4. A 5 day culture of rabbit lymph node. In toto preparation. Fibroblasts form a broad area in the fibrin and surround the newly formed fine fibers and the dense fibers of the explant. Foot's silver method. 65×.

6 Day Cultures.

In the interior of the explanted tissue are large anastomosing fibers which tend to form bundles. Very fine fibrils interweave among the large fibers. Often the fibers are arranged in a whorl about clear spaces that appear as fenestrations in the complex network. The large fibers that tend to form bundles stain differently than heretofore, they have a purplish-black hue resembling that of collagenous fibers. They are more or less straight and not wavy. In the outer zone of the culture are many migrating fibroblasts and macrophages, among which are very fine fibrils as described above in the 5 day cultures (fig. 5).

7 Day Cultures.

Within the explanted tissue the fibers consist principally of large, wavy or coiled, serpentine bundles. There is a fine network of fibrils between the large bundles of fibers. The bundles stain a blackish-brown with the silver. In the region of the new formed fibers surrounding the explant ,the growth has become very dense.

Many macrophages and fibroblasts have migrated into the outer sector of the culture; between them is an open network of fibrils which extends into the fibrin beyond the migrating cells.

8 Day Cultures.

At this stage the fibers have become very numerous and highly developed. The reticulum of the explanted tissue has become a network of small bundles of collagenous fibers. The network of fine

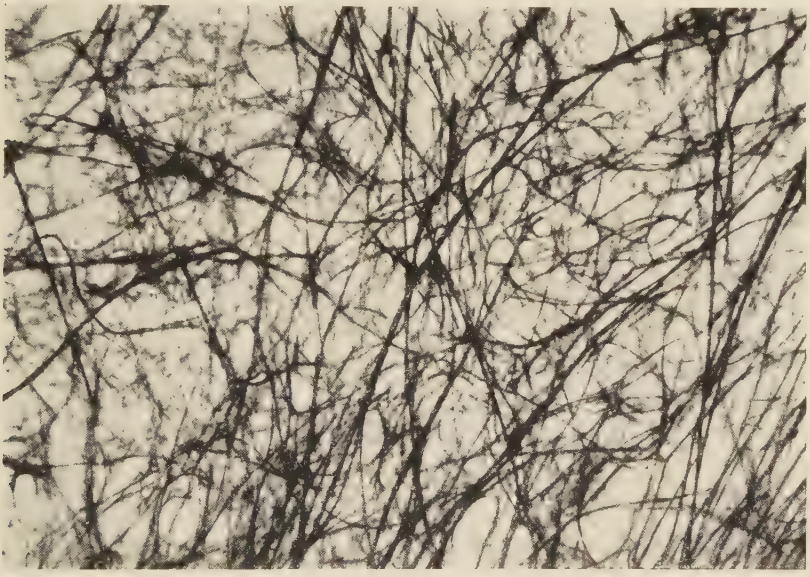


Fig. 5. A 6 day culture of rabbit lymph node. In toto preparation. The explanted reticulum fibers have become collagenous and a fine network of fibrils has developed between them. Foot's silver method. 640×.

argyrophil fibrils in the zone of new growth is also beginning to stain with silver, like collagenous fibers.

The marginal zone of the area of new growth consists of macrophages and fibroblasts. The migrating cells furthest from the explant are large macrophages. Many mitotic figures are seen throughout the culture. The fibroblasts are surrounded by a basket-work of fine fibrils. This fine network extends into the fibrin and is also continuous with the dense network of the zone of new growth near the explant.

9, 10, and 11 Day Cultures.

By this time considerable liquefaction has taken place and the central portions containing the explant have fallen out. The zone of new growth remains attached to the cover slip. Immediately surrounding the clear area of the missing explant is a dense mass of fibers that stain suggestively like collagenous fibers. This zone is fringed with a network of argyrophil fibers and migrating cells.

Serial Sections.

These sections were stained by Foot's modification of Bielschowsky's silver impregnation method and counterstained by the Azan method. Because of the thickness of the in toto preparations, it was found necessary to prepare serial sections of cultures fixed at the same time as those used for the in toto preparations. Each method has certain advantages which will be discussed below.

Adult Rabbit Lymph Node Control.

The reticulum fibers stain black with silver and form a network in the meshes of which are many lymphocytes, hemocytoblasts and reticulum cells (see fig. 1, plate 2).

5 Hour Cultures.

At this stage the sections resemble very closely those of the lymph node controls. The reticulum fibers form an open network. The cells are very numerous in the spaces surrounded by the reticulum fibers. They are predominantly lymphocytes and a relatively small number of reticulum cells. Practically no migration of cells has taken place at this time. There is no change in either the reticulum fibers throughout the sections or in the small bit of collagenous tissue of the trabecula which is present in a few sections of the culture.

1 Day Cultures.

In the interior of the explant, the reticulum fibers stain black with silver and form an open network, the spaces of which are filled with numerous cells. Many of these are pyknotic and stain very deeply with the silver and Azan stains. A few cells have

wandered out from the explant and have begun to hypertrophy. There is no change in the reticulum fibers.

2 Day Cultures.

The reticulum of the explant has become curly and fewer cells are in its meshes. Many lymphocytes have migrated from the explant and have become elongated and irregular in shape; within the explant are many cells of this type and many with pyknotic nuclei. Just beyong the explanted tissue are typical fibroblasts with large oval nuclei which contain fine granules of chromatin. The fibrin network is clearly defined; it has a grayish blue color.

3 Day Cultures.

The reticulum of the explanted tissue is shredded so that it appears as if its fibers have frayed out to produce a very fine network of fibrils between the original fibers. Throughout the explant are many lymphocytes, macrophages with large black granules in their protoplasm, and fibroblasts with their clear cytoplasm stretched out into many processes. Many fibroblasts and macrophages have wandered out from the explant. In the zone of new growth the cells are surrounded by a very fine, delicate network of silver fibrils. These fibrils appear as fine, stippled, black lines and cannot be confused with the fibrin network which stains a grayish blue; nor can they be confounded with the protoplasmic processes of the macrophages and fibroblasts which stain a clear pale blue. The arrangement of the new formed argyrophil fibrils is often very much like that of the fibrin network and it seems as if they have used it as a scaffolding. The entire formation described here is not in one flat plane, as can be clearly seen with a binocular microscope.

4 Day Cultures.

In the interior of the explant the reticulum consists of curly, frayed, black, silver impregnated fibers, between which there is a network of very fine, black fibrils (fig. 2, plate 2). The fibers and fibrils are often arranged around oval or rounded areas which are more or less free of fibrile and contain cells. These are lymphocytes, macrophages and fibroblasts. In addition, there are many degenerating cells and much cellular debris. In one of the cultures a piece of the capsule of the lymph node has been explanted. It stains a deep blue

and consists of large wavy bundles of collagenous fibers; these are localized in one area of the sections. Many cells have migrated from the explant. The majority of them are lymphocytes, some are macrophages and fibroblasts. In this area of new growth argyrophil fibrils have appeared; they now form a more definite, fibrillar network than was present in the 72 hour specimens. The general direction of the fibrils extends radially from the explant. The network of fibrils is denser near the explant. The outermost layer of the zone of new growth consists of migrating cells among which are no argyrophil fibers.

5 Day Cultures.

In these sections a most spectacular change has taken place. The explant up to this time has shown a dense network of black argyrophil fibers and fibrils; it now presents a distinct, dense network of deep, blue stained fibers and fibrills. The latter are not arranged in large wavy bundles but are fine and more or less straight like the argyrophil fibers. Mingled with these blue stained fibers are frayed bundles of large and small argyrophil fibers (fig. 3, plates 3). Thus, there exist here fibers that are in part argyrophil and in part acidophil and the former condition is giving away to the latter. The size and arrangement of these argyrophil fibers is identical with that of the acidophil ones. The type of cell that is predominant in this unique network of fibers is the fibroblast. In the zone of new growth are many fibroblasts and macrophages. The fibroblasts are numerous large, spindle shaped cells and are packed close together. Between them fine argyrophil fibers form a delicate network. Far into the fibrin a few fibroblasts have migrated. They are more or less isolated so that the entire cell can be seen in detail. There are no fibers out in this remote zone.

6 Day Cultures.

The explant has become a dense network of deeply blue stained bundles of collagenous fibers. Some of the bundles are more or less straight while others are wavy (fig. 4, plate 3). Most of the zone of new growth consists of a dense network of blue stained collagenous fibers and fibrils. There are still some argyrophil fibers in this region. In the peripheral portion of the zone of new growth are fine, black, argyrophil fibrils forming a deli-

cate network. These sections, in general, show a marked increase in the formation of collagenous fibers and a simultaneous disappearance of argyrophil fibers.

7 Day Cultures.

In the interior of the explant are large wavy bundles of collagenous fibers and small straight collagenous fibers that interweave with one another so as to form a dense, tangled network. The zone of new growth consists of intermingled, fine, straight, collagenous fibers and argyrophil fibers. The general direction of the fibers is radiating from the explant. The fibers are very close to-gether and the fenestrations have become oval slits. There are no fibrils in the fibrin beyond the migrating cells.

8, 9, 10, and 11 Day Cultures.

The cultures of these stages present practically the same picture. Considerable liquefaction has taken place so that the explants have fallen out, leaving large, clear, rounded areas surrounded by the zone of new growth. The fibers immediately around the large clear area of the missing explant are concentrically arranged; further out they are arranged radially. They are mostly fine, blue fibers; intermingled with them are some black, argyrophil fibers. To-gether they make up a dense network of fibers. Its meshes contain very many macrophages with their cytoplasm packed with phagocytized material. There are no fibers in the fibrin beyond the migrating cells.

Summary and Discussion.

New argyrophil fibers appear in both the explant and new growth of adult rabbit lymph node after 3 days incubation. The argyrophil fibers at first develop most abundantly in the explanted tissue; from the beginning they anastomose freely and form a delicate network. In 5 day cultures the transformation of reticulum fibers into collagenous begins, again first in the explanted tissue.

In the explanted tissue new reticulum fibers grow from the original reticulum fibers. Here the substance of individual reticulum fibers often becomes separated so that the fiber becomes apparently larger and longitudinally striated. Delicate fibrils seem to fray out from these and produce a fine secondary network within their

meshes. In some cases the new fibrils grow directly out from the explanted reticulum fibers into the new growth.

It thus seems that the presence of reticulum fibers favors the production of new argyrophil fibers. For in Maximow's cultures of blood leucocytes, no reticulum fibers were explanted and the first argyrophil fibers appeared in 25 day cultures. In his thymic cultures, which were also from a reticular tissue, he obtained new reticulum fibers in his youngest cultures (12 days). Maximow found the first typical collagenous fibers appearing in his 25 day thymic cultures.

From my experiments we may conclude that there exists in tissue cultures of reticular tissue, a condition that stimulates both the formation of new argyrophil fibers and their transformation into collagenous fibers within about 5 days.

Argyrophil fibers are a precursor of collagenous fibers both in the body and in tissue cultures. In the body, reticulum fibers normally exist as such throughout life in the reticular tissue. However, in the repair of inflammatory lesions of reticular tissue, abundant collagenous fibers develop from the reticulum fibers. It is possible that explanted lymphoid tissue is subjected to conditions similar to those which obtain when it is inflammed in the body. For a rapid transformation of reticulum fibers into collagenous fibers also takes place in the cultures.

The experiments reported here are in favor of Foot's view, that there is a chemical difference between reticulum and collagenous fibers. I have found fine argyrophil fibrils and fine collagenous fibrils lying side by side. I have also found large, black, silver stained fibers and large, deeply blue stained fibers of the same size; often a single fiber is in part silver stained and in part stained blue with anilin blue. In addition, bundles of argyrophil fibers and bundles of collagenous fibers of the same proportions are often interwoven in a network (see plate 3, fig. 3). I cannot explain the difference on the basis of Mallory's theory that reticulum fibers are merely separated collagenous fibers.

The present views on the relation of reticulum fibers to reticulum cells is that the fibers lie either within the cell or along its cytoplasm. In these cultures I cannot decide in favor of either view. During the stage of the formation of new argyrophil fibers, in both the explant and the new growth, the cells are mainly lymphocytes and macrophages with some fibroblasts. The macrophages are often seen with a network of reticulum fibers about them and

it is possible that they may influence the production of the fibers. The newly formed argyrophil fibrils in the zone of new growth radiate from the explant. In the older cultures they seem pulled to-gether as if the movement of cells through them exerts a tension on them.

During the stage of the transformation of the argyrophil fibers into collagenous fibers the predominant type of cell is the fibroblast. It would thus appear that the fibroblast influences collagenous fiber formation. In the cultures an increase in the development of collagenous fibers is attended by an increase in the number of fibroblasts. It is by no means proved, however, that the fibroblasts are the only cells which are concerned in the formation of collagenous fibers.

A few, isolated, spirochete-like, argyrophil fibrils appear here and there in the cultures beyond the zone reached by cells. They are not as numerous nor do they form a network as in Maximow's thymus cultures. Their exact nature is unknown. Maximow concluded that these isolated fibers fused and that they were forerunners of the dense networks of argyrophil fibers which developed in his thymic cultures. All of my lymph node cultures were fixed some days before the youngest of Maximow's thymic cultures. The few spirochete-like structures in the fibrin in my cultures played no part in the formation of the dense networks of reticulum fibers which formed in both the explant and the zone of newly formed tissue.

Conclusions.

1) New argyrophil fibers appear in the explants of cultures of rabbit lymph node in 3 days.

2) The newly formed reticulum fibers in the explants are always connected with the explanted fibers.

3) Similar fibers appear at about the same time in the zone of new formed tissue in these cultures.

4) These argyrophil fibers in the explant turn into collagenous fibers during the fifth day in culture.

5) During the sixth day the newly formed reticulum fibers in the migration zone beyond the explant change into collagenous fibers.

6) The differences between reticulum and collagenous fibers are not merely of physical nature.

I am indebted to Dr. W. Bloom for the aid he has given me in carrying out this investigation.

Chicago, August 14, 1929.

References.

1. Baitsell, G. A., A study of the development of connective tissue in Amphibia. Amer. Journ. Anat., Vol. 28, p. 447, 1920—21.
2. — The origin and structure of a fibrous tissue which appears in cultures of adult frog tissue. Journ. Exp. Med., Vol. 21, p. 455, 1915.
3. — Origin of the connective tissue ground substance in the chick embryo. Quart. Journ. of micr. Sci., Vol. 69, p. 571, 1925.
4. Foot, N. C., Chemical contrasts between collagenous and reticular connective tissue. Amer. Journ. of Path., Vol. 4, p. 525, 1928.
5. — and Mènard, M. C., A rapid method for the silver impregnation of reticulum. Arch. path. and lab. Med., Vol. 4, p. 211, 1927.
6. Harrison, R., The development of the balancer in Amblystoma, studied by the method of transplantation and in relation to the connective tissue problem. Journ. Exp. Zool., Vol. 41, p. 349, 1925.
7. Laguesse, E., La structure lamelleuse et le dévéloppement du tissu conjonctif lâche chez les mammifère en général et chez l'homme en particulier. Arch. de Biol., T. 31, p. 173, 1921.
8. Lewis, M. R., Development of connective tissue fibers in tissue cultures of chick embryos. Contrib. to Embr. Carnegie Inst. of Washington, Vol. 6, No. 17, p. 47, 1917.
9. Mallory, F. B., A hitherto undescribed fibrillar substance produced by connective tissue cells. Journ. of med. Research, Vol. 10, p. 334, 1904.
10. — and Parker, F., Reticulum. Amer. Journ. Path., Vol. 3, p. 515, 1927.
11. Maximow, A., Tissue cultures of young mammalian embryos. Carnegie Inst. of Washington Publ., Contrib. to Embr., Vol. 16, p. 47, 1927.
12. — Cultures of blood leucocytes. From lymphocyte and monocyte to connective tissue. Arch. f. exp. Zellforsch., Bd. 5, S. 169, 1928.
13. — Ueber die Entstehung von argyrophilen und kollagenen Fasern in Kulturen von Bindegewebe und von Blutleukozyten. Centralbl. f. allg. Path. und path. Anat., Bd. 43, S. 145, 1928.
14. — Bindegewebe und blutbildende Gewebe. Handbuch der mikroskopischen Anatomie des Menschen. Herausgegeben von W. v. Möllendorff. Bd. 2, T. 1, S. 232, 1927.
15. — Ueber die Entwicklung argyrophiler und kollagener Fasern in Kulturen von erwachsenem Säugetiergewebe. (Nach des Verfassers Tode geschrieben und veröffentlicht von Dr. W. Bloom.) Zeitschr. f. mikr.-anatom. Forschung, Bd. 17, H. 3—4, S. 625, 1929.

16. Nageotte, J., L'organisation de la matière dans ses rapports avec la vie. Paris, Librairie Félix Alcan, 1922.

17. Plenk, H., Über argyrophile Fasern (Gitterfasern) und ihre Bildungszellen. Ergebn. d. Anat. u. Entwicklungsgeschichte, Bd. 27, S. 302, 1927.

Legends for Plates.

The figures of plates I and II are drawn with the camera lucida; Bausch and Lomb 1.9 mm. oil immersion objective and 10× ocular; tube length of 150 mm. They are all stained with Foot's silver method and Azan.

Plate 2.

Fig. 1. Section of adult rabbit lymph node. Shows the arrangement of the reticulum fibers and the types of cells present. Most of these are small lymphocytes; there are a few reticulum cells and hemocytoblasts. Compare with text figure 1.

Fig. 2. Section from the explant of a 4 day culture of adult rabbit lymph node. This illustrates how the explanted fibrous reticulum appears to fray to form new argyrophil fibrils. The network is irregular and is broken at places by holes relatively free of fibers. There are a few lymphocytes and macrophages.

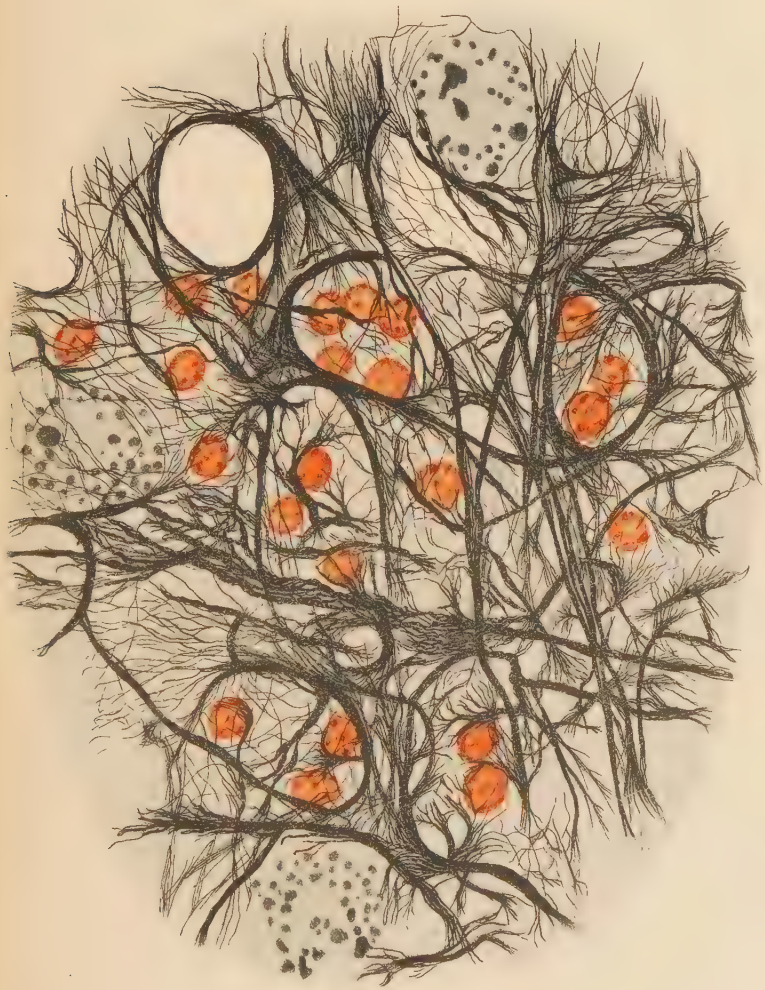
Plate 3.

Fig. 3. Section of a 5 day culture. Here reticulum and collagenous fibers of the same size are intimately intermingled. Some fibers are partly argyrophyl and partly acidophil (i. e. show collagenous transformation). The cells are mostly fibroblasts.

Fig. 4. Section of a 6 day culture. At this stage heavy bundles of wavy collagenous fibers have formed. The fiber network conceals the cells.

1.

2.



Lith. Anst. v. F. A. Funke, Leipzig

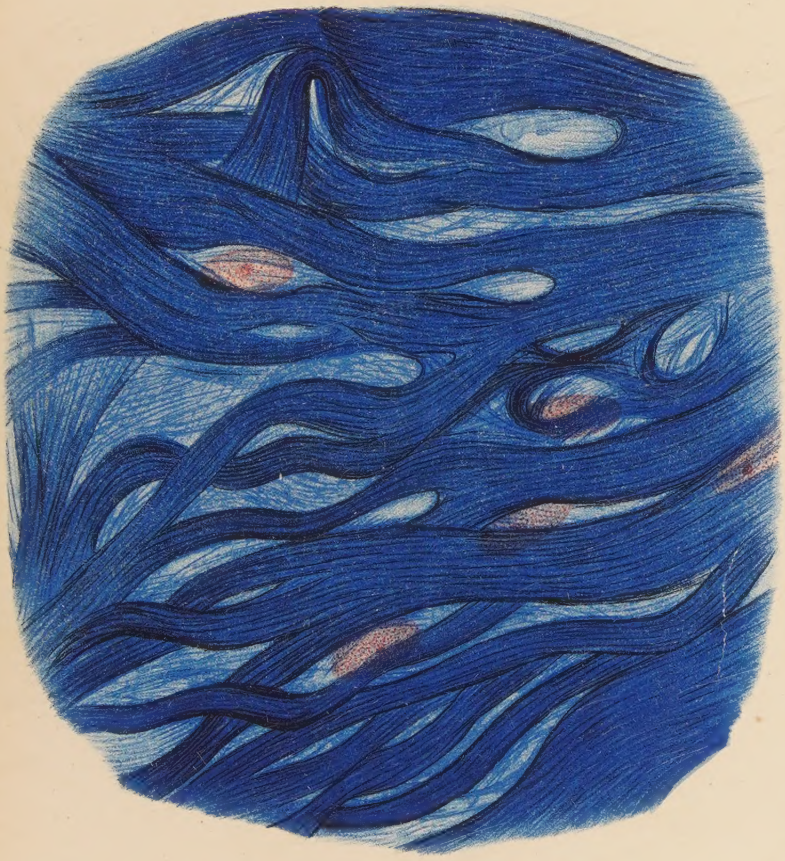
Jena

3.

Mc Kinney

Verlag

4.



Lith. Anst. v. E. A. Funke, Leipzig.

her in Jena